The Hantavirus Glycoprotein G1 Tail Contains Dual CCHC Type Classical Zinc Fingers

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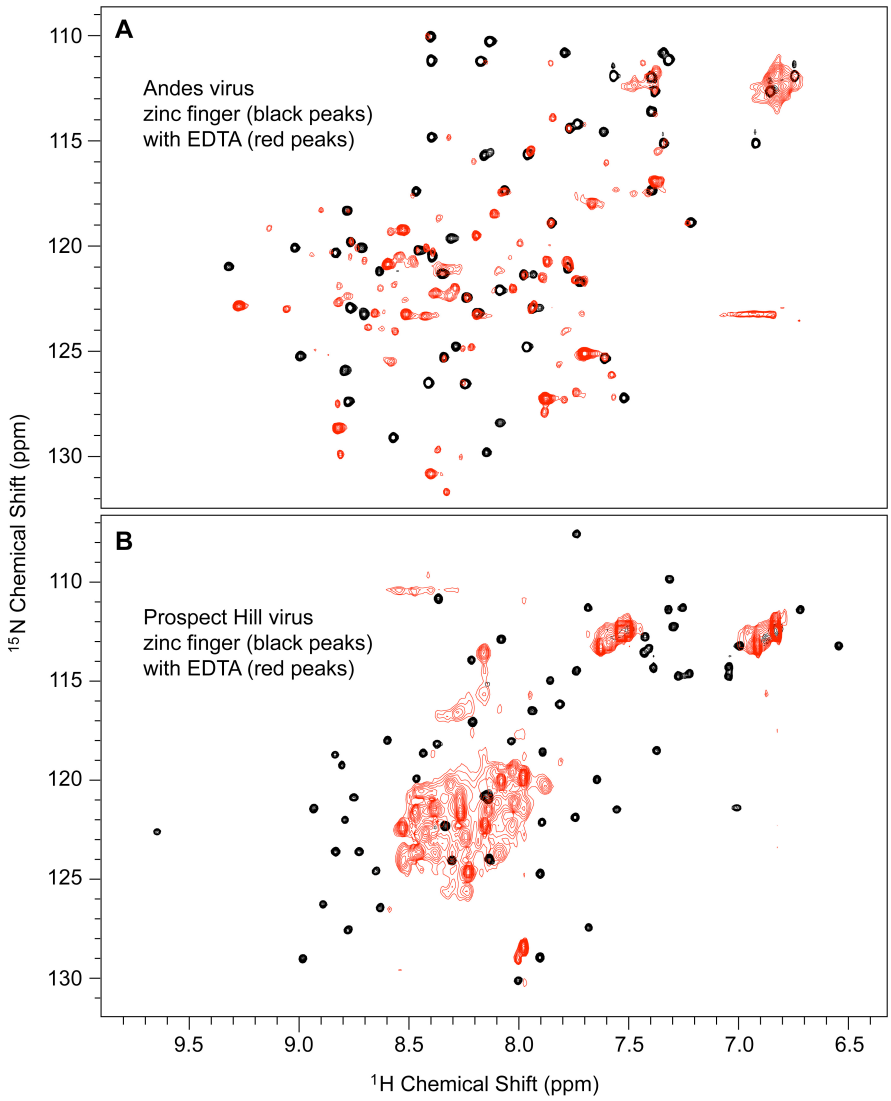

Fig. S1. The proper folding of the hantavirus G1 tail zinc finger domain depends on Zn^{2+}-binding. ^{1}H-^{15}N HSQC spectra of **(A)** Andes virus and **(B)** Prospect Hill virus G1 zinc finger domains before (black peaks) and after (red peaks) addition of 4 mM EDTA. In the Andes zinc finger, addition of EDTA resulted in the deterioration of the HSQC peaks, with peaks that are sharp and other peaks with reduced intensities, as well as collapse of asparagine and glutamine side chain resonances. In the Prospect Hill virus, addition of EDTA collapsed the backbone and side chain peaks (at 7.8-8.6 and 6.8-7.6 ^{1}H ppm, respectively) into a characteristic HSQC of an unfolded protein. We determined the NMR structure of the G1 zinc finger domain from the Andes virus, which is pathogenic to humans (whereas Prospect Hill virus is not).

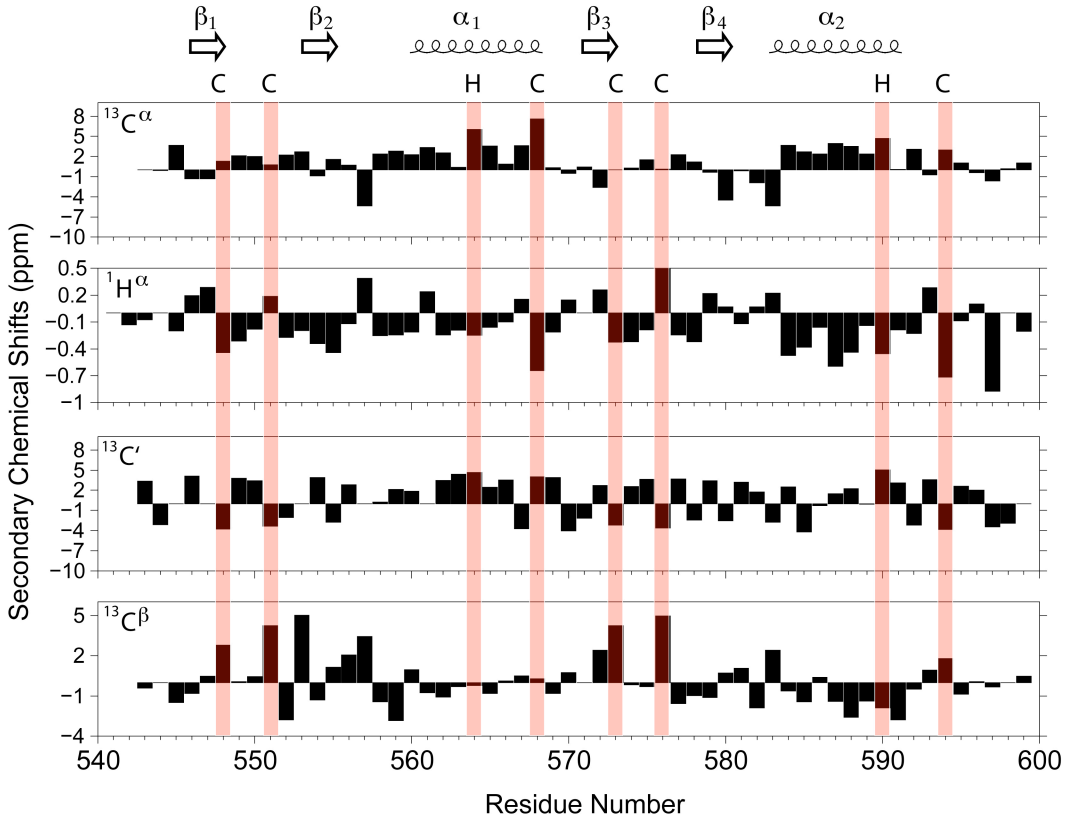

Fig. S2. Secondary C^{α}, H^{α}, C', and C^{β} chemical shifts of the Andes virus G1 zinc finger domain. Shown are the CCHC-zinc coordination ligands (red bars) and the secondary structures (β-strands and α-helices).

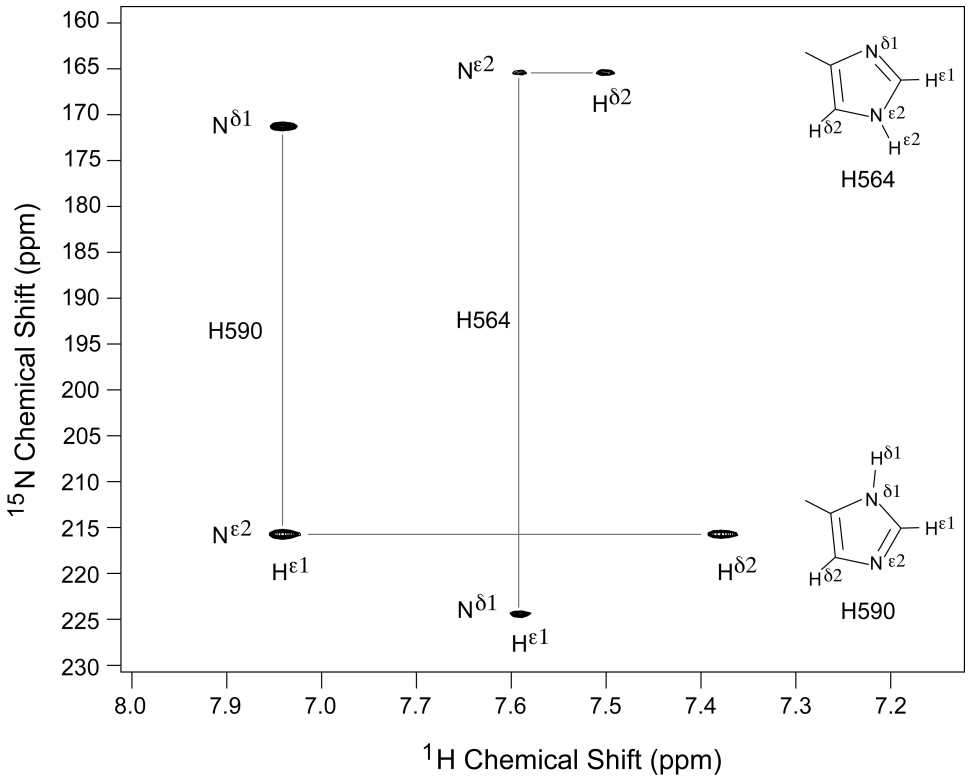

Fig. S3. The tautomeric states of the Zn^{2+}-coordinating histidines (His^{590} and His^{564}) were determined by 2D ^{1}H-^{15}N HMQC following the method of Pelton *et al*. (*Protein Sci.*, 1993, 2, 543-558). This spectrum was acquired using an ^{15}N-labeled protein in buffer (10 mM $NaPO_4$ pH 7.0, 10 mM NaCl, 1 mM DTT, 0.1 mM $ZnSO_4$) in 100% D_2O with the following acquisition parameters: ^{15}N carrier frequency (195 ppm), ^{15}N sweep width (70 ppm), number of scans (32), number of ^{15}N complex points (128).

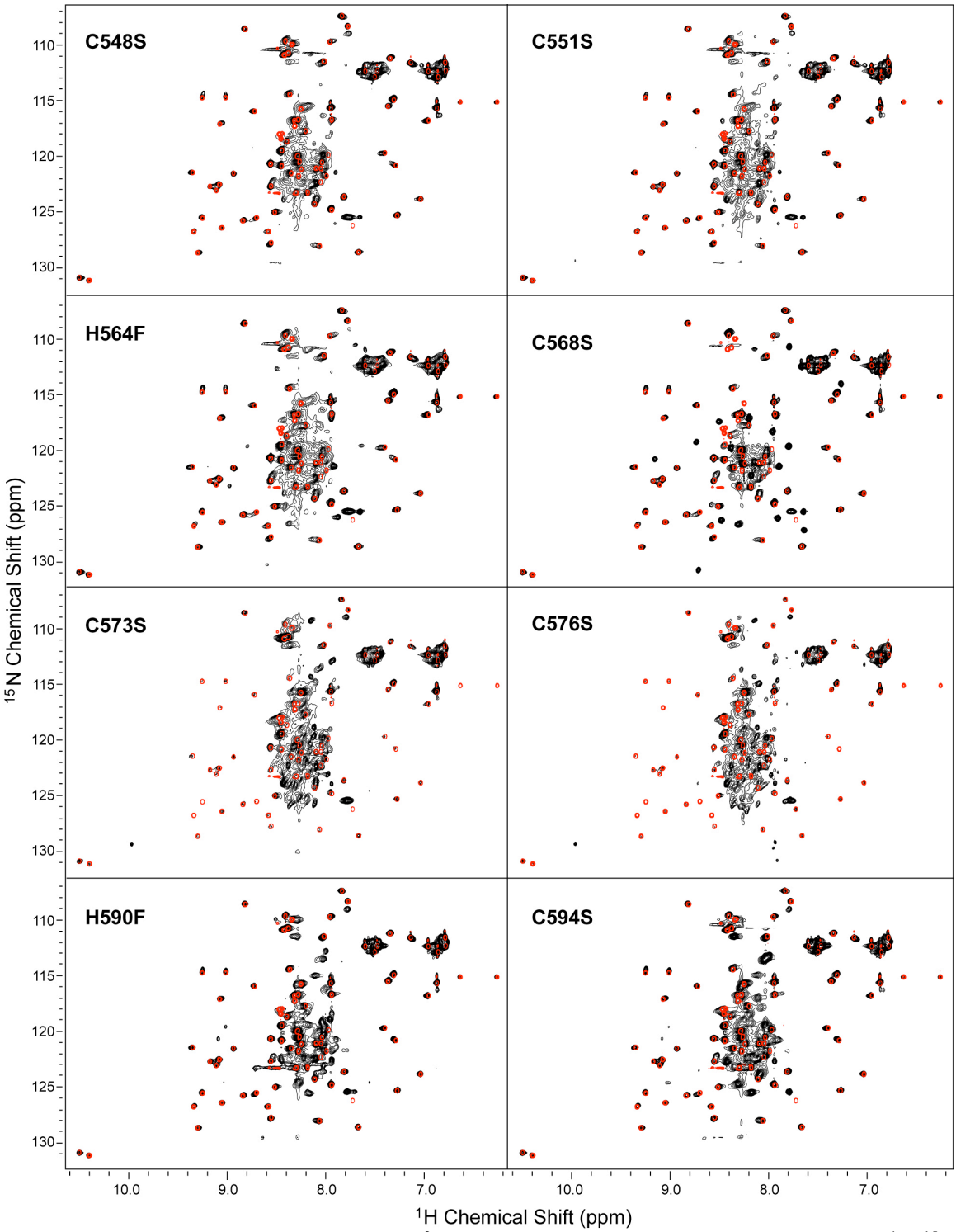

Fig. S4. Effect of point mutations in the Zn^{2+}-coordinating cysteine and histidine residues. ^{1}H-^{15}N HSQC spectra of GB1-zinc finger domain fusion proteins with point mutations in the eight Zn^{2+}-coordinating residues. Cysteine was mutated into serine and histidine into phenylalanine. Spectra of GB1 fusion proteins (black peaks) are overlayed with the spectrum of free GB1 tag (red peaks).

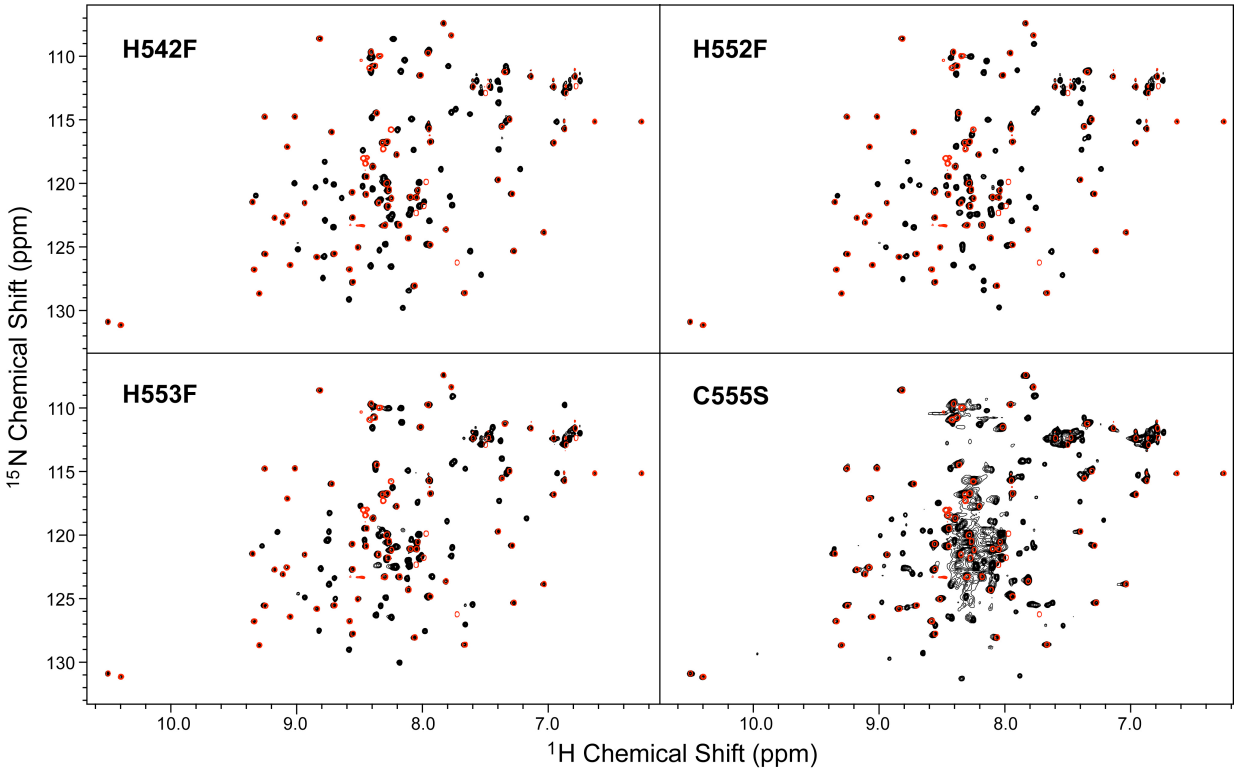

Fig. S5. Effect of point mutation in histidine and cysteine residues that do not coordinate Zn^{2+} ion. The 2D ^{1}H-^{15}N HSQC spectra of histidine point mutants (H542F, H552F, and H553F) showed the characteristic well dispersed and distinct peaks of a folded protein. The spectrum for the C555S mutant also showed well dispersed peaks, however, there is a region in the middle of the spectrum that is characteristic of an unfolded protein. C555 is a non-Zn^{2+}-coordinating residue, however, it forms part of the hydrophobic core, and the C555S mutation caused an unfolding in some regions of the zinc finger domain. Shown are the ^{1}H-^{15}N HSQC spectra of GB1-zinc finger fusion proteins (black peaks) overlayed with the spectrum of free GB1 tag (red peaks).

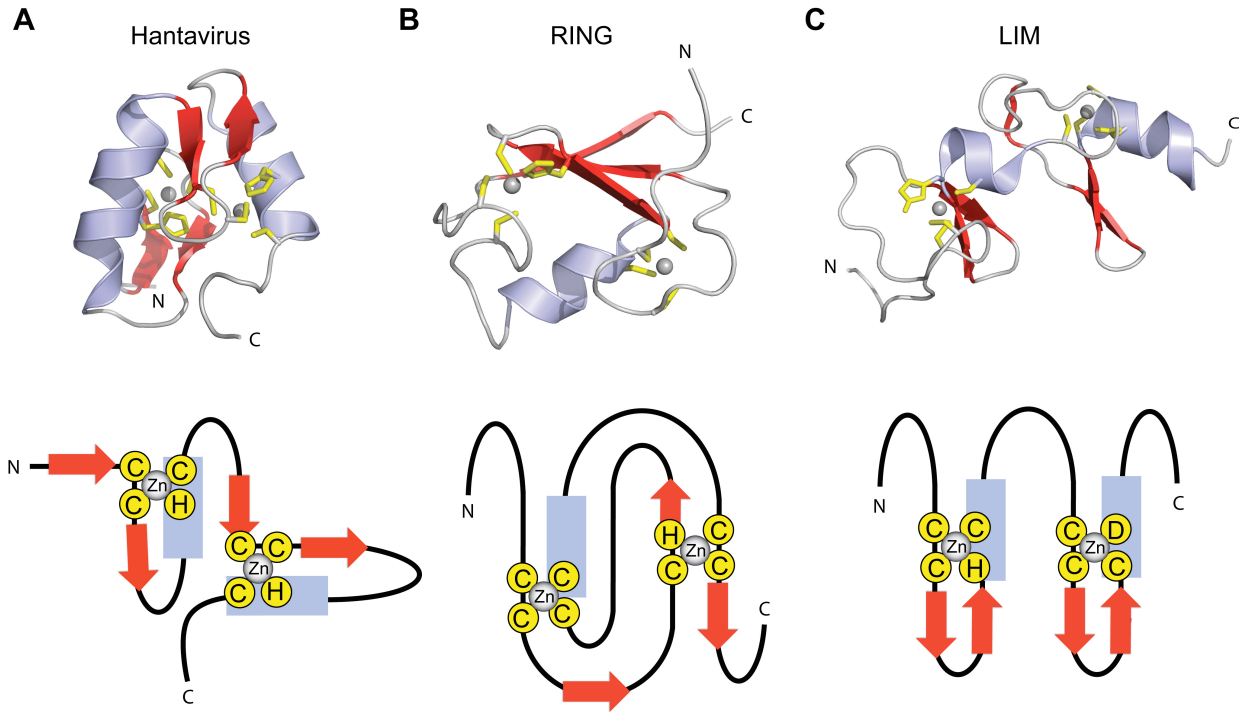

Fig. S6. The structures (top panel) and zinc binding topology (bottom pane) of the **(A)** Andes virus G1 zinc finger domain is different from that of **(B)** RING domain (PDB 1CHC) and (C) LIM domain (PDB 1M3V). The cartoon depiction of zinc binding topology was adapted from Gamsjaeger *et al*. (*Trends Biochem. Sci.*, 2007, 32, 63-70).

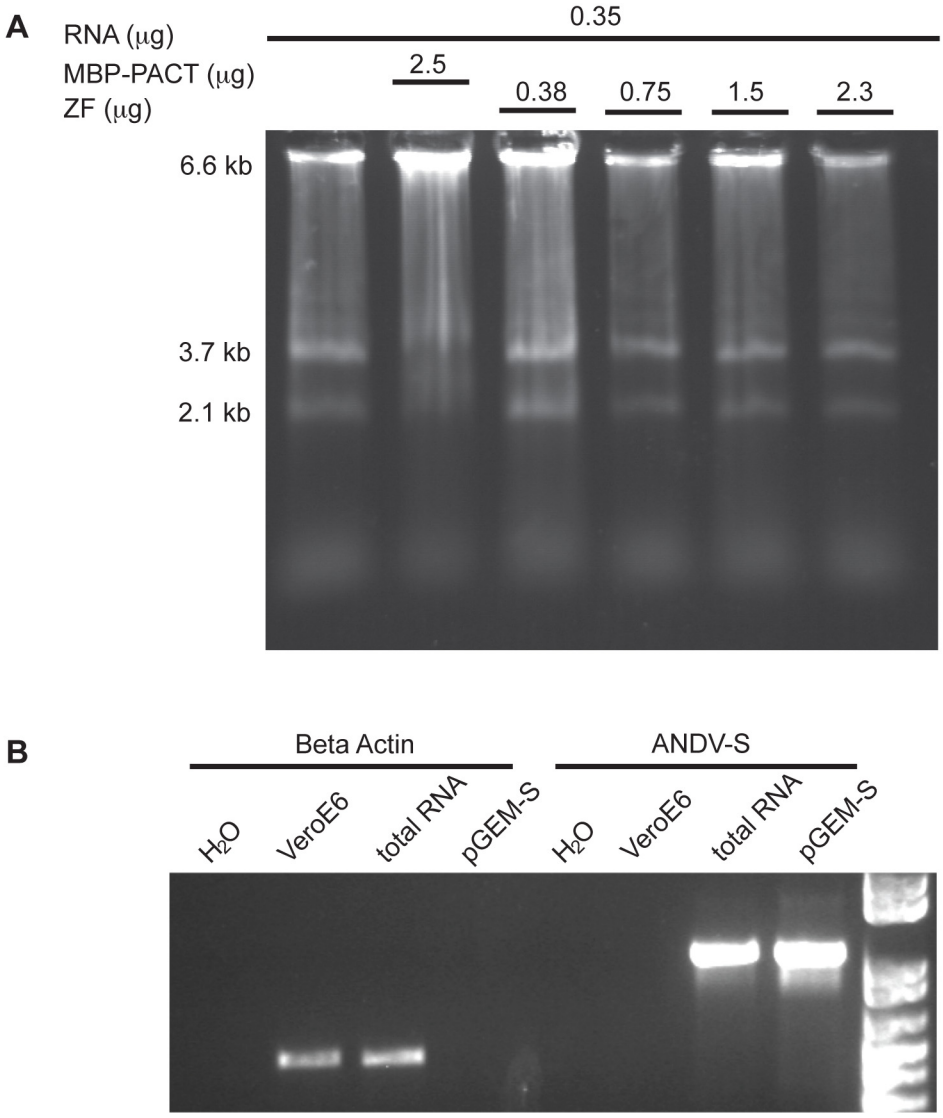

Fig. S7. The hantaviral zinc finger domain does not bind RNA. **(A)** Electrophoretic mobility shift assay of total RNA (from VeroE6 cells 14 days post-infection with Andes virus), a known RNA-binding protein (PACT) expressed as a fusion protein with the maltose binding protein (MBP), and the Andes virus zinc finger domain (ZF). The smearing of the RNA bands by MBP-PACT showed nonspecific RNA binding, while the RNA bands remained unchanged with the zinc finger domain. **(B)** RT-PCR using primers specific to beta actin and the S-segment of the Andes virus genome (ANDV-S) confirmed the presence of cellular and viral RNA. Controls were uninfected VeroE6 cells, water, and plasmid DNA with the S segment of the Andes virus genome (pGEM-S).